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EFFECT OF DOUBLE-BONDS ON BIMOLECULAR FILMS IN MEMBRANE MODELS

GERHARD LAGALY [a*], ARMIN WEISS [b] and EVA STUKE [b]

[a] *Institut für Anorganische Chemie der Universität Kiel, Olshausenstrasse 40/60, 23 Kiel, and* [b] *Institut für Anorganische Chemie der Universität München, Meiserstrasse 1/3, 8 München 2 (G.F.R.)*

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Summary

The effect of unsaturation (especially by *cis*-bonds) is studied on bimolecular films of saturated and unsaturated alkylammonium ions and alkanols between silicate surfaces as model systems for lipid layers in membranes. Three types of structures are observed: all-*trans*-blocks, kink-blocks and *gauche*-blocks. The knowledge of the sequence of these phases and their thermal transitions provides detailed deductions about the role of double-bonds. *cis*-Unsaturated chains are taken up in bimolecular films as isomers with *cis-trans-gauche* conformation. This conformation makes the shape of the chain similar to that of kinked chains (chains with *gauche-trans-gauche* (—) conformation) and enables the incorporation into the film without greater sterical hindrance. The experimental results are in good agreement with X-ray measurements on biological membranes by Engelman (Engelman, D.M., J. Mol. Biol. 47, 115—117 (1970) and 58, 153—165 (1971)).

Increasing the concentration of *cis*-chains decreases the transition temperature of the kink-blocks into *gauche*-blocks. The variation of the transition temperature with concentration of *cis*-unsaturated chains in the model system is similar to that observed for *Escherichia coli* membranes. It is suggested that phase changes in biomembranes are of the same nature: transition of kink-block analogues as ordered phases into *gauche*-block assemblies as less ordered phases.

Introduction

Biological membranes contain in their lipid entire characteristic amounts of unsaturated long chain compounds. Configuration (*cis-trans*) and position of the double-bonds within the chains have strong influence on the properties.

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* Correspondence to be addressed to Professor Dr. Gerhard Lagaly.

[1—3]. Thermal transitions are controlled by the ratio of cis to trans-unsaturation. Detailed information could be obtained with mutants of *Escherichia coli* auxotropic for unsaturated fatty acids [4—11]. The results support the assumption that phase transitions of the lipid entire of biological membranes are similar to transitions in thin lipid films [3,12] and influence not only permeability and properties of the lipid region but, in turn, also characteristic membrane-protein dependent functions.

By X-ray diffraction on biological membranes it could be established that the thickness of the lipid entire decreases at the transition points [13—15]. Detailed information on the nature of these phase transitions and their structural background is unavailable due to the difficulties of X-ray investigations on biological material. Some conceptions about the structural rearrangements could be obtained from investigation of the thermal transitions in thin lipid films [3,12,16—19]. Our model provides detailed studies of possible phase transitions of the lipid entire.

Model System

The model consists of a bilayer of long chain compounds sandwiched between the silicate sheets of mica-type layer silicates. These silicates consist of negatively charged silicate sheets of about 10 Å thickness which are stacked parallel above each other and separated by exchangeable interlayer cations (Na^+, Ca^{2+}). Bimolecular films between the sheets are formed by replacing the inorganic interlayer cations with alkylammonium ions and subsequent intercalation of long chain alkanols. Since the crystalline order is not destroyed, X-ray measurements of the basal spacings give the thickness of the unit: bimolecular film + silicate sheet. Any variation of the structure which entails a variation in its thickness is monitored by a variation of the basal spacing. Simple long chain compounds (Table I) as film-forming compounds are chosen to avoid the greater complexity of glycerides and phospholipids (Fig. 1).

TABLE I

COMPOSITION OF THE BIMOLECULAR FILMS

Mol% *cis*-chains	Mol per mol beidellite			
	Stearyl-ammonium ions	Oleyl-ammonium ions	Stearyl alcohol	Oleyl alcohol
0	0.4	—	1.6	—
20	0.4	—	1.2	0.4
	—	0.4	1.6	—
40	0.4	—	0.8	0.8
	—	0.4	1.2	0.4
60	0.4	—	0.4	1.2
	—	0.4	0.8	0.8
80	0.4	—	—	1.6
	—	0.4	0.4	1.2
100	—	0.4	—	1.6

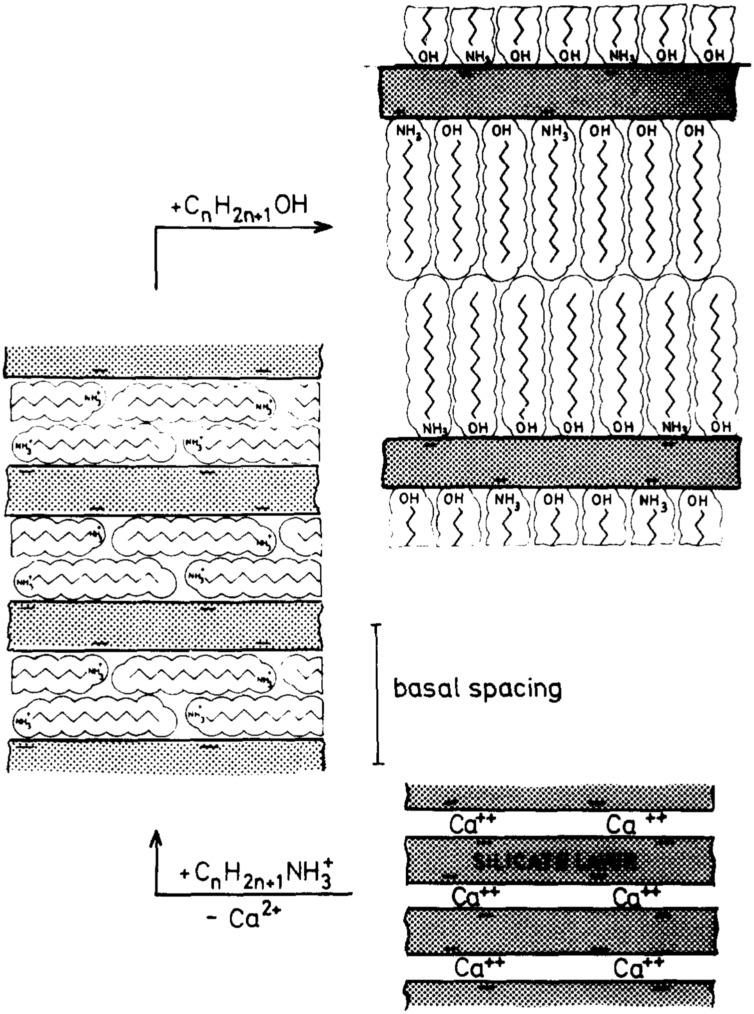


Fig. 1. Bimolecular films between silicate sheets as models for the lipid entire of phospholipid membranes.

Experimental

Starting material is the natural mica-type layer silicate beidellite with Ca^{2+} between the silicate layers. After exchange of stearyl- or oleylammonium ions for Ca^{2+} in aqueous solution the alkanol complexes were prepared as previously described [20].

As a consequence of the cation exchange the proportion of alkylammonium cations in the film is constant. Per formula unit of beidellite there are 0.43/2 Ca ions which are quantitatively exchanged by stearyl- or oleylammonium ions. 1.6 molecules of alkanol are intercalated per unit, so that the film contains stearyl (or oleyl) ammonium ions and alkanol molecules in the ratio 0.43:1.6. If mixtures of alkanols are applied, it is assumed that the proportion of alkanols in the interlayer is about the same as in the mixture (Table I). This is a rough

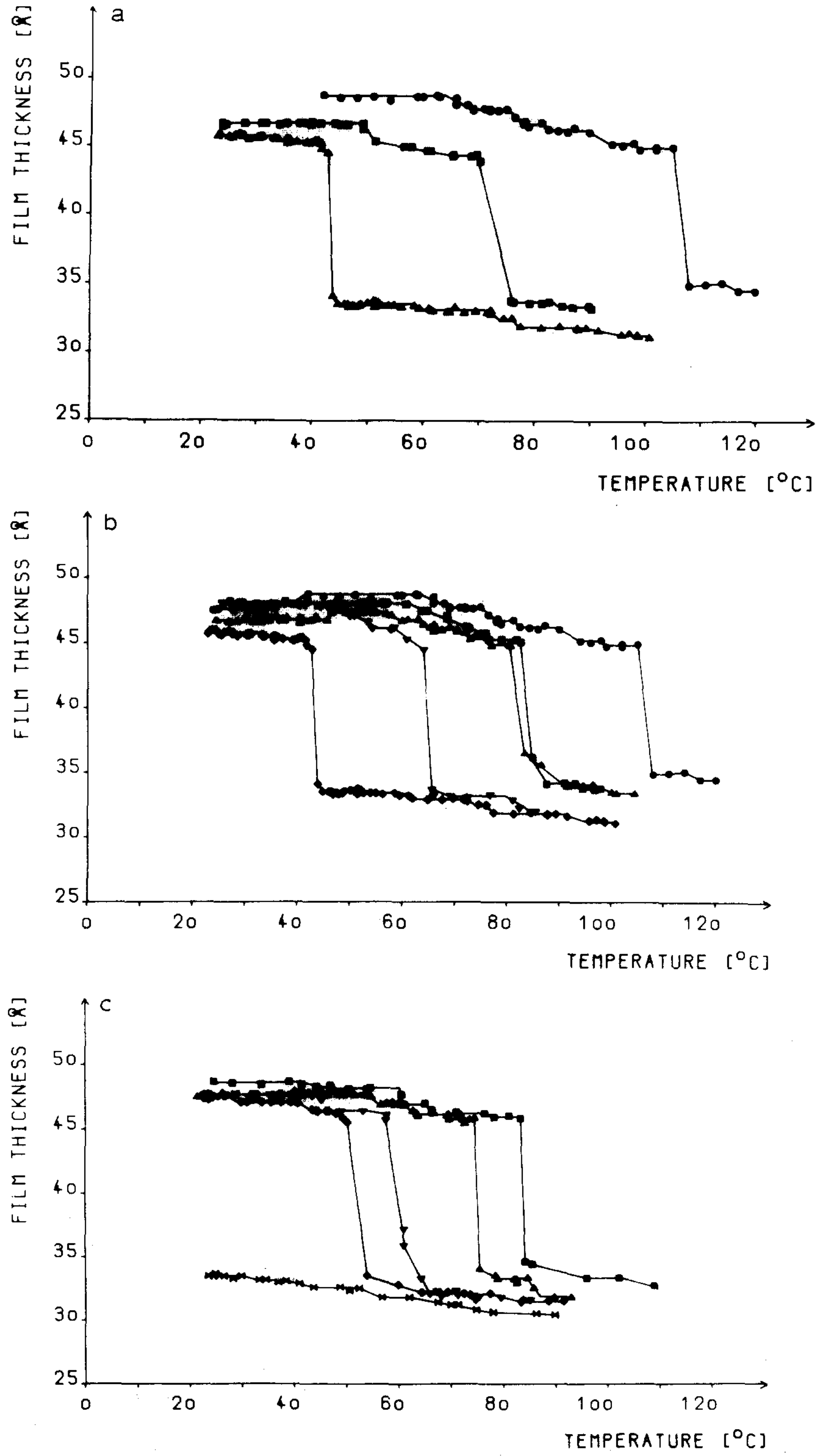


Fig. 2. (a) Variation of film thickness with temperature. Composition of the film (mol per mol beidellite): 0.4 stearylammonium ions + 1.6 stearyl alcohol (●), + 1.6 elaidyl alcohol (■), + 1.6 oleyl alcohol (▲). (b) Variation of film thickness with temperature. Composition: stearylammonium ions + stearyl alcohol + oleyl alcohol (see Table I): ●, 0% *cis*; ■, 20% *cis*; ▲, 40% *cis*; ▼, 60% *cis*; ◆, 80% *cis*. (c) Variation of film thickness with temperature. Composition: oleylammonium ions + stearyl alcohol + oleyl alcohol (see

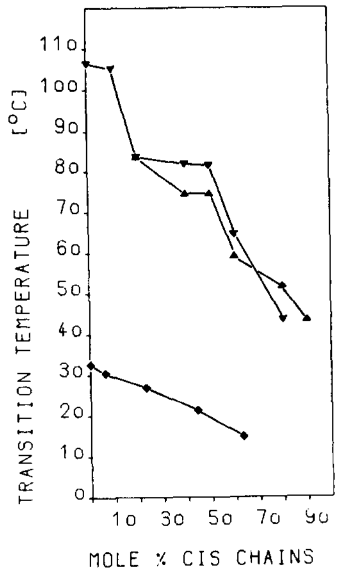


Fig. 3. Transition temperature vs. concentration of *cis*-unsaturated chains. Model system: ▼, film with stearylammonium ions; ▲, film with oleylammonium ions. Biomembrane: ◆, *E. coli* membrane (K 1062) [6].

approximation, but exact experimental data are difficult to obtain.

The basal reflexions ((00*l*)-reflexions) of the beidellite · alkanol complexes were measured between 20 and 120°C [20,21]. The heating and cooling cycles were repeated several times under different conditions, especially heating and cooling rates. The d_{00l}-values of the (00*l*)-reflexions were converted to the basal spacing d_L ($d_L = 1d_{001} = 2d_{002} \cdots = ld_{00l}$ ($l \leqslant 8$)). d_L minus the thickness of the silicate layer (9.5 Å) gives the thickness of the bimolecular film.

Results

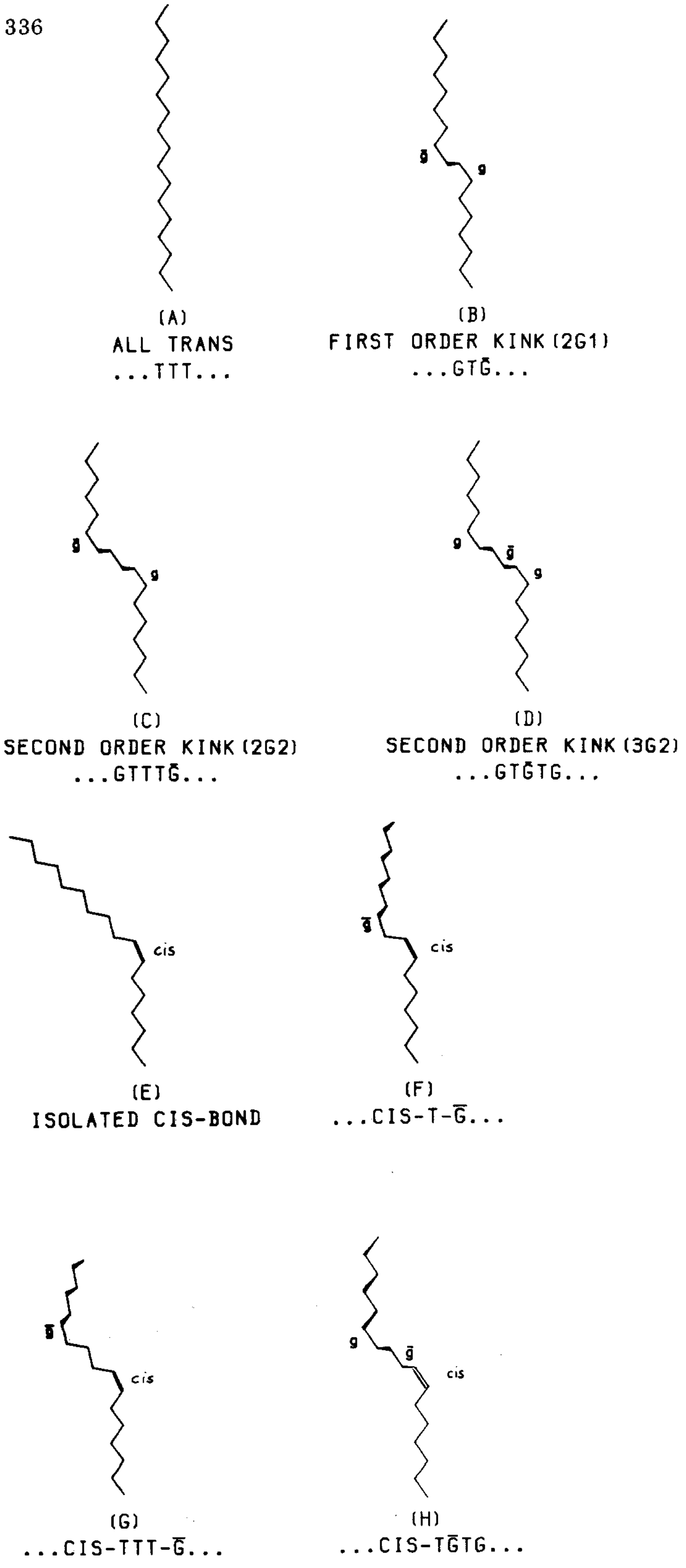
Fig. 2 reports the variation of the thickness of the bimolecular film. The curves are reproducible and independent upon the temperature scans. Sometimes hysteresis effects could be observed on heating and cooling cycles. The thickness is drastically reduced within a narrow temperature range. Below and above the transition points ($T_{\beta/\alpha}$) it decreases in steps of 0.5—1.2 Å. The different forms of the bimolecular film below $T_{\beta/\alpha}$ are described as β-phases, those above $T_{\beta/\alpha}$ as α-phases [20,21].

The transition temperature decreases with increasing unsaturation (Figs. 2 and 3). The decrease of $T_{\beta/\alpha}$ by *cis*-bonds is most pronounced for 0—20 and > 50 mol % *cis*-chains and is small for 20—50 mol %. If all chains in the film are *cis*-chains, $T_{\beta/\alpha}$ falls below room temperature.

Discussion

Kink-block and gauche-block model

The variation of film thickness with temperature in the model is best interpreted by cooperative formation of kinks as defects of alkyl chains and the



(A)
ALL TRANS
...TTT...

(B)
FIRST ORDER KINK (2G1)
...GT$\bar{G}$...

(C)
SECOND ORDER KINK (2G2)
...GTTT$\bar{G}$...

(D)
SECOND ORDER KINK (3G2)
...GT$\bar{G}$TG...

(E)
ISOLATED CIS-BOND

(F)
...CIS-T-$\bar{G}$...

(G)
...CIS-TTT-$\bar{G}$...

(H)
...CIS-T$\bar{G}$TG...

Fig. 4. Conformation of all-*trans* alkyl chains, kinked chains (nomenclature by Pechhold [22]) and *cis*-unsaturated chains.

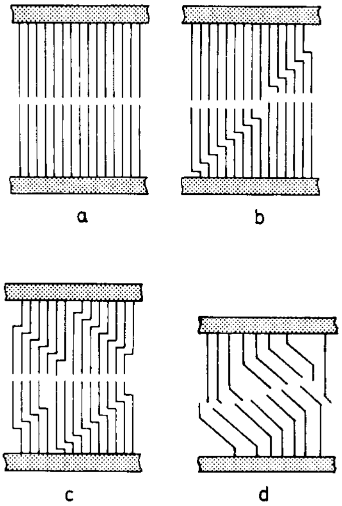


Fig. 5. Kink and *gauche*-blocks. (a), all-*trans* chains (phase β_1); (b), alternating blocks of all-*trans* chains and kinked chains (phase β_2); (c), alternating blocks of kinked chains (phase β_3); (d) *gauche*-blocks.

rearrangement of the kinked chains to kink-blocks (Fig. 4) [22]. β-Phases below $T_{\beta/\alpha}$ represent different kink-blocks which differ in the number of kinks per chain or in the order of the kinks (Fig. 5). Since an increasing number of kinks per chain or increasing order of kinks shortens the overall-length of the chain by 1.27 Å or multiples of it, the β-phases are distinguished by their thickness (Table II). If the number of kinks per chain exceeds a critical value [20, 21,22], the high temperature α-phases with different structure appear. *

Effect of cis-bonds

The long axes of the chain parts on each side of a *cis*-bond include an angle of 130° (Fig. 4). It is difficult for this conformation to be adopted by chains which are assembled in bimolecular films. Insertion of a *gauche*-bond, however, leads to conformations similar to kinked chains, and enables the chains to be incorporated into kink-blocks without greater sterical interference (Figs. 4,6). This explains the observation of Engelman [14] that *cis*-bonds do not prevent dense-packing of the chains in lamellar phases.

Chains with *cis*-*t*-$\bar{g}$-conformation can directly act as nuclei for kink-block formation. It is therefore easily understood that the thickness of films containing *cis*-chains also decreases in sharp steps with rising temperature. The most favored kink-blocks should be of the type shown in Fig. 6c, which corresponds to β_3-phases (46.5 Å thick, Table II). However, β_1 and β_2-phases are also observed.

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* The decrease of the film thickness is too high to be explained by formation of an increased number of 2*g*1-kinks per chain. This interpretation would also contrast to the observation that the decrease of the film thickness at $T_{\beta/\alpha}$ depends upon the lateral chain arrangement and the attainable interlayer chain density [21,24]. Presumably the chains contain isolated *gauche*-bonds, *gtg*-conformations [22] or high order kinks. The sharp basal reflexions in the X-ray diagram prove a very uniform film thickness though the chains are less ordered, more mobile [23] and looser packed than in the β-phases (33—38 Å^2/chain; in the kink-block structures: 20—25 Å^2/chain). An idealized model is shown in Fig. 5d.

TABLE II

STABILITY OF KINK-BLOCK (β) AND *GAUCHE*-BLOCK (α) PHASES OF SATURATED AND UNSATURATED BIMOLECULAR FILMS

Results given for percent *cis*-chains

	Thickness	Thermal stability of stearylammonium beidellite (°C)				
		0	20	40	60	80
β_1	about 48.5 Å	—65	—57	—	—	—
β_2	about 47.5 Å	70—75	?	—57	—48	—
β_3	about 46.5 Å	79—90	?	57—63	≈50	—
β_4	about 45.5 Å	93—105	75—83	67—80	54—62	—40
α	34.5—35.5 Å	>107	>84	>90	>65	>45

	Thickness	Thermal stability of oleylammonium beidellite (°C)				
		20	40	60	80	100
β_1	about 48.5 Å	—55	—	—	—	—
β_2	about 47.5 Å	60—65	—54	—45	—37	—
β_3	about 46.5 Å	67—77	56—61	49—54	43—48	—
β_4	about 45.5 Å	78—83	67—72	≈57	≈50	—
α	34.5—35.5 Å	>84	>72	>57	>50	>25

With low *cis*-contents (up to 20 mol% *cis*-chains) the film thickness is the same as in the corresponding β_1-phases of saturated films (Table II). Evidently, most of the saturated chains have the all-*trans* conformation and determine the thickness of the film (Fig. 6a). With enhanced *cis*-content (up to 60 mol%) the thickness decreases only by 1.1 Å, indicating the formation of β_2-phases. Combinations of *cis*-chains in one monolayer with all-*trans* chains in the other monolayer should be very abundant. Such distribution of kinked and all-*trans* chains makes the structure less regular and creates more misfits between the chains (Fig. 6b). Its stability requires a sufficient number of pairs of *cis*-chains and all-*trans* chains *. This is ensured up to about 67 mol% of *cis*-chains. At higher concentrations the number of these pairs becomes too low and the structure collapses into β_3-analogues or α-phases.

Even if the β_ω-phases (mostly $\omega = 3$) of *cis*-unsaturated films are of the same kind as those of saturated films (Fig. 5), the transition temperatures $T_{\beta/\alpha}$ largely decrease with increasing concentraion of *cis*-chains. At first it may be inferred that the geometry of a *cis*-*t*-$\bar{g}$ conformation differs somewhat from the *g*-*t*-$\bar{g}$ conformation and the packing of *cis*-chains with kinked chains cannot be optimal. But another structural aspect appears to be more efficient. Dense-packing of kinked chains in kink-blocks requires the chains to be kinked in different positions (Fig. 5); the displacement by the kinks proceeds diagonally through the bimolecular film. In *cis*-chains the combination *cis*-*t*-$\bar{g}$ corresponding to the kink conformation *g*-*t*-$\bar{g}$ is fixed at distinct positions in the chain.

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* Assemblies as shown in Fig. 6b may be of importance for our understanding of kink-block structures. Their formation will be favored by an entropy increase due to the mixing of kinked and all-*trans* chains.

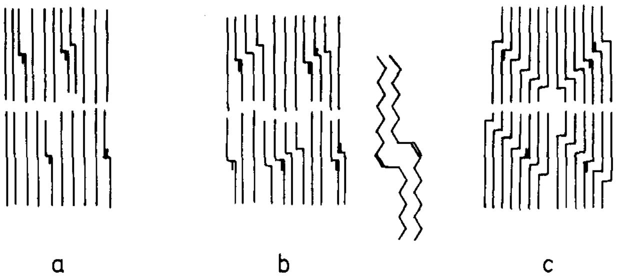

Fig. 6. Structure of bimolecular films with *cis*-unsaturated chains. (a), *cis*-chains as defects in all-*trans* chain assemblies (β_1-analogues up to 20% *cis*-chains); (b), mixing of all-*trans*, *cis*-unsaturated and kinked chains in the β_2-analogues; (c), incorporation of *cis*-chains into β_3-kink-blocks.

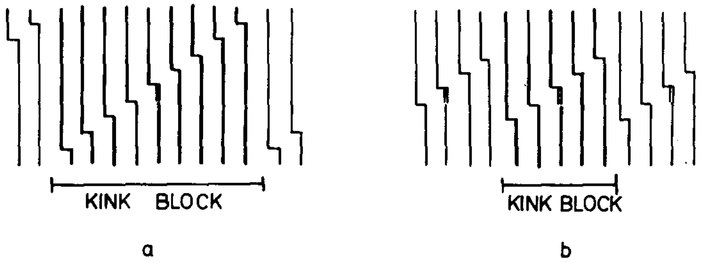

Fig. 7. Influence of *cis*-chain concentration on the size of the kink-blocks at low (a) and high (b) concentration of *cis*-chains.

With low concentration of *cis*-chains the dimension of the kink-block remains unchanged, the kink-block being built around the *cis*-chain (Fig. 7). With increasing concentration the *cis*-chains prevent the formation of fully extended kink-blocks (Fig. 7) because they have the kink-like conformations at false positions. The *cis*-chains act as defects in the structure and decrease the size of the kink-block. The kink-block becomes smaller with increasing content of *cis*-chains and the number of misfits at the kink-block borders increases. As a consequence the thermal stability decreases and the arrangement into α-phases is shifted to lower temperatures.

Effect of trans-bonds

The geometry of trans-unsaturated alkyl chains differs not much from all-*trans* saturated chains. The chains can be incorporated in bimolecular films without larger sterical hindrance. Since a *trans*-bond cannot rearrange into a kink, the kink-blocks with high concentrations of unsaturated chains are less extended or contain additional voids between kinked unsaturated chains. As a consequence the reduced kink-block size decreases the temperature of transition.

Conclusions

The model system shows in detail the influence of *cis*- and *trans*-double bonds on phase transitions in bimolecular films of long chain compounds. The

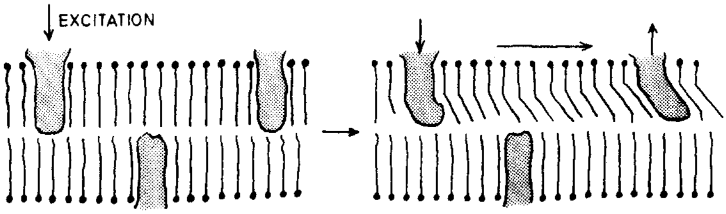

Fig. 8. A schematic model for information transfer within the lipid layer by cooperative phase transitions.

temperature of the β/α-transition is largely influenced by unsaturation. The same behavior has been reported for biological membranes (*E. coli* membranes [6,8,10]). Due to the different kinds of the film forming long chain compounds and their presumably higher order in the model system, the transition temperatures are higher than in biological membranes, but their variation with increasing *cis*-content is very similar to that of *E. coli* membranes [6] (Fig. 3). *

The importance of kinks and *gauche*-bonds for bilayer structures has been accentuated by Seelig and Niederberger [17], Nagle [26], Scott [27], McCammon and Deutsch [28] and Jackson [29]. In contrast to these models the experiments reported demonstrate that the nature of the phase transition from an ordered into a less ordered phase [7,10,30–34] is not a rearrangement of a structure with a small concentration of kinks into one with large kink concentrations but a cooperative transition of kink-blocks into different structures (*gauche*-blocks) whose film thickness lies markedly below the values expected for chains with the highest number of kinks. The decrease of the lipid layer thickness as reported by Engelman based on careful X-ray investigations on biomembranes [13,14] is of the same order of magnitude as expected for formation of *gauche*-blocks. Träuble [35] first discussed the movement of small molecules across membranes in terms of kinks. The activation energy of about 4.7 kcal/mol for the movement of kinks in kink-blocks of the model system deduced from NMR-measurements [23] is in agreement with Träuble's theory [35].

The *gauche*-blocks with less ordered chains and smaller chain packing density evidently require smaller activation energies for tranport processes across the lipid layer than the more ordered kink-block phases. The enhanced mobility of chains or chain parts in the *gauche*-blocks with activation energies below 5 kcal/mol could be confirmed by NMR measurements [23]. This corresponds to the reduced activation energies of transport processes in biomembranes above the transition points [6,10].

The ability of the lipid layer to undergo phase transitions turns out to be of great importance for interactions of proteins with the lipid layer [36]. Lateral phase separation [7,30] and protein aggregation above certain temperatures, as postulated by Heerikhuizen at al. [37], require not only high lateral mobilities of the lipid molecules but also distinct phase transitions. Short mention should

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* The stepwise decrease of film thickness with temperature, clearly observed in the model system, is not expected to be observable in biomembranes or in lipid films on water. It has been shown [21, 22,24,25] that the appearance of kink-blocks in the form of stepwise decrease of the film thickness requires special lateral arrangements of the chains which are not fullfilled in the case of biomembranes or lipid films.

also be made of another consequence. Because of its cooperative nature the phase changes are pronounced mechanisms for information transfer within the membrane from one point of excitation (probably from protein localized in the lipid layer) to an acceptor far away (Fig. 8).

References

1 Van Deenen, L.L.M. (1968) in Regulatory Functions of Biological Membranes (Järnefeld, J., ed.), pp. 72—86, Elsevier, Amsterdam

2 Demel, R.A., Geurts van Kessel, W.S.M. and van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 266, 26—40

3 Blok, M.C., van Deenen, L.L.M. and de Gier, J. (1976) Biochim. Biophys. Acta 433, 1—12

4 Schairer, H.U. and Overath, P. (1969) J. Mol. Biol. 44, 209—214

5 Overath, P., Schairer, H.U. and Stoffel, W. (1970) Proc. Natl. Acad. Sci. U.S. 67, 606—612

6 Overath, P., Hill, F.F. and Lamnek-Hirsch, I. (1971) Nat. New Biol. 234, 264—267

7 Overath, P., Brenner, M., Gulik-Krzywicki, T., Shechter, E. and Lettelier, L. (1977) Biochim. Biophys. Acta, in press

8 Wilson, G., Rose, S.P. and Fox, C.F. (1970) Biochim. Biophys. Res. Commun. 38, 617—623

9 Esfahani, M., Barnes, E.M. and Wakil, S.J. (1969) Proc. Natl. Acad. Sci. U.S. 64, 1057—1064

10 Esfahani, M., Limbrik, A.R., Knutton, S., Oka, T. and Wakil, S.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3180—3184

11 Rosen, B.P. and Hackette, S.L. (1972) J. Bacteriol. 110, 1181—1189

12 Overath, P. and Träuble, H. (1973) Biochemistry 12, 2625—2634

13 Engelman, D.M. (1970) J. Mol. Biol. 47, 115—117

14 Engelman, D.M. (1971) J. Mol. Biol. 58, 153—165

15 Kreutz, W. (1972) Angew. Chem. 84, 597—614

16 Sackmann, E. and Träuble, H. (1972) J. Am. Chem. Soc. 94, 4492—4498

17 Seelig, J. and Niederberger, W. (1974) Biochemistry 13, 1585—1588

18 Gally, H.U., Seelig, A. and Seelig, J. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1447—1450

19 Rand, R.P., Chapman, D. and Larsson, K. (1975) Biophys. J. 15, 1117—1124

20 Lagaly, G., Fitz, S. and Weiss, A. (1975) Clays Clay Min. 23, 45—54

21 Lagaly, G. (1976) Angew. Chem. Int. Ed. Engl., 15, 575—586

22 Pechhold, W., Liska, E., Grossman, H.P. and Hägele, P.C. (1976) Pure Appl. Chem. 46, 127—134

23 Stohrer, M. and Noack, F. (1975) Progr. Colloid Polymer Sci. 57, 61—68

24 Fernandez-Gonzales, M., Weiss, A., Beneke, K. and Lagaly, G. (1976) Z. Naturforsch. 31 b, 1205—1211

25 Baur, H. (1975) Progr. Colloid Polymer Sci., 58, 1—18

26 Nagle, J.F. (1975) J. Chem. Phys. 63, 1255—1261

27 Scott, H.O. (1975) Biochim. Biophys. Acta 406, 329—346

28 McCammon, J.A. and Deutsch, J.M. (1975) J. Am. Chem. Soc. 97, 6675—6681

29 Jackson, M.B. (1976) Biochemistry 15, 2555—2561

30 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271—2275

31 Cupp, J., Klymkowski, M., Sands, J., Keith, A. and Snipes, W. (1975), Biochim. Biophys. Acta 389, 345—357

32 Stockton, G.W., Johnson, K.G., Butler, K.W., Polnaszek, C.F., Cyr, R. and Smith, I.C.P. (1975) Biochim. Biophys. Acta 401, 535—539

33 Verma, S.P., Wallach, D.F.H. and Schmidt-Ulrich, R. (1975) Biochim. Biophys. Acta 394, 633—645

34 Schirmer, H., Hegner, D., Schnepel, G.H. and Wellhörner, H.H. (1975) Biochim. Biophys. Acta 394, 93—101

35 Träuble, H. (1971) J. Membrane Biol. 4, 193—208

36 Papahadjopoulos, D., Moscarello, M., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317—335

37 Van Heerikhuizen, J., Kwak, E., van Bruggen, E.F.J. and Wiltholt, B. (1975) Biochim. Biophys. Acta 413, 177—191